

Effects of phosphate availability on the root system architecture: large-scale analysis of the natural variation between *Arabidopsis* accessions

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ABSTRACT

Developmental plasticity is one main adaptative response of plants to the availability of nutrients. In the present study, the naturally occurring variation existing in *Arabidopsis* for the growth responses to phosphate availability was investigated. Initially details of the effects of phosphate starvation for the four currently used accessions Cvi, Col, Ler and Ws were compared. A set of 10 growth parameters, concerning the aerial part and the root system, was measured in both single-point and time-course experiments. The length of the primary root and the number of laterals were found to be consistently reduced by phosphate starvation in all four accessions. These two robust parameters were selected to further screen a set of 73 accessions originating from a wide range of habitats. One-half of the accessions showed also a reduced primary root and less lateral roots when phosphate-starved, and 25% were not responsive to phosphate availability. For the last quarter of accessions, phosphate starvation was found to affect only one of the two growth parameters, indicating the occurrence of different adaptative strategies. These accessions appear to offer new tools to investigate the molecular basis of the corresponding growth responses to phosphate availability.

Key-words: *Arabidopsis*; accessions; natural variation; phosphate availability; root system architecture.

INTRODUCTION

Re-orientation of growth constitutes one of the main adaptative responses of plants to changes in nutrient availability. For numerous plant species, this leads to an increased growth of roots relative to the aerial part. As a general consequence, large alterations of the root system architecture are observed (Drew 1975), although different kinds of modifications occur depending on the nature of the

limiting nutrient and on the plant species. Changes reported in *Arabidopsis* concern, for instance, the growth of the primary root in phosphate-starved plants (Williamson *et al.* 2001), the growth of lateral roots in nitrate-, phosphate- or iron-starved plants (Moog *et al.* 1995; Zhang & Forde 1998; Williamson *et al.* 2001), the density of lateral roots in phosphate- or iron-starved plants (Moog *et al.* 1995; Williamson *et al.* 2001), or the formation of root hairs in phosphate- or iron-starved plants (Bate & Lynch 1996; Schmidt & Schikora 2001), whereas phosphate starvation promotes the formation of proteoid roots in *Lupinus albus* (Johnson, Carroll & Allan 1996), or affects the growth angle of roots in *Phaseolus vulgaris* (Bonser, Lynch & Snapp 1996) while simultaneously altering the growth and density of lateral roots (Borch *et al.* 1999).

Up to now, the search for the genes controlling such growth responses to nutrient availability was based mainly on genetic studies of mutants. In *Arabidopsis*, an important success was the discovery of the MADS box transcription factor *ANRI*, involved in the adaptative increase in lateral root elongation in nitrate-rich regions (Zhang & Forde 1998). Selective involvement of hormones in the growth responses to nitrate, phosphate and iron availability was also demonstrated from the behaviour of mutants affected in their sensitivity to specific hormones (Schmidt & Schikora 2001; Williamson *et al.* 2001; Linkohr *et al.* 2002; Lopez-Bucio *et al.* 2002; Al-Ghazi *et al.* 2003). On another hand, relatively little attention was paid to the variation occurring naturally among accessions as an alternative way to search for genes having large effects in growth responses (Alonso-Blanco & Koornneef 2000). A notable exception in *Arabidopsis* concerns the characterization of phosphate acquisition efficiency, a parameter which includes, among others, growth responses to phosphate availability (Narang, Bruene & Altmann 2000; Narang & Altmann 2001). In these studies quite large variations were observed between accessions. However, due to the limited number of accessions that were characterized, it was not possible to decide whether these variations corresponded to more or less pronounced responses among accessions, or revealed the occurrence of different adaptative strategies.

The present study was aimed at investigating the extent

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of the biodiversity of growth responses to phosphate starvation in *Arabidopsis*, with the goal of identifying accessions showing contrasted responses and, eventually, different growth adaptative strategies. For this purpose, the behaviour of four accessions that are widely used in genetic studies was first characterized in details. Two of the growth parameters displaying the more robust responses to phosphate starvation were further selected, and measured among a set of 73 accessions originating from a wide range of habitats.

MATERIALS AND METHODS

Plant material

Arabidopsis thaliana accessions (Table 1) were progenies from NASC (<http://nasc.nott.ac.uk>) and provided by Dr Gerrit Beemster (Department of Plant Genetics, University of Gent, Belgium), with the exception of accession Ws-4 (obtained from INRA, Versailles, France).

Chemicals

CaSO₄, KH₂PO₄ and H₃Bo₃ were from Merck-Eurolab (Darmstadt, Germany); KNO₃, MES, MnCl₂ and CuCl₂ were from Sigma-Aldrich (St Louis, MO, USA); NaFe-EDTA and NH₄Mo were from Fluka (Buchs, Switzerland); Bactoagar was from Difco (Detroit, MI, USA). All other reagents were of analytical grade.

Plant culture

Seeds of each accession were first sterilized under stirring for 15 min in 20 mL of 50% (v/v) ethanol and 4% (w/v) bayrochlor[®] (Bayrol, Planegg, Germany) and then washed three times in 100% ethanol and three times in sterile H₂O. The sterile seeds were carefully sown on 12 cm × 12 cm Petri dishes (Greiner Bio-one, Frickenhausen, Germany) containing the culture medium (CM) and 1 mM KH₂PO₄ (termed below as 'normal culture medium'). The CM had the following composition: 0.5 mM CaSO₄, 2 mM KNO₃,

Table 1. Overview of the *Arabidopsis thaliana* accessions investigated. NASC numbering is given according to <http://nasc.nott.ac.uk>

Accession	Origin	NASC number	Accession	Origin	NASC number
Aa-0	Aua/Rhön, Germany	N935	In-0	Innsbruck, Austria	N1239
An-1	Antwerpen, Belgium	N945	Is-0	Isenburg, Germany	N1241
Bd-0	Berlin, Germany	N963	Jl-1	Jelinka, Czech Republic	N1249
Be-0	Bensheim, Germany	N965	Jm-0	Jamolice, Czech Republic	N1259
Bl-1	Bologna, Italy	N969	Kä-0	Kärnten, Austria	N1267
Bla-1	Blane, Spain	N971	Kil-0	Killean, England	N1271
Blh-1	Bulhary, Czech Republic	N1031	Kin-0	Kindalville, USA	N1273
Bs-1	Basel, Switzerland	N997	Kondara	Tajikistan	N916
Bur-0	Burren, Eire	N1029	Ler-1	Landsberg, Poland	N1642
C24	Coimbra, Portugal	N906	Ll-0	Llagostera, Spain	N1339
Ca-0	Camberg, Germany	N1061	Ma-0	Marburg, Germany	N1357
Can-0	Canary Islands	N1065	Mv-0	Martha's Vineyard, USA	N1387
Chi-0	Chisdra, Russia	N1073	Nd-0	Niederzenz, Germany	N1391
Co-0	Coimbra, Portugal	N1085	Nok-0	Noordwijk, Netherlands	N1399
Col-0	Landsberg, Poland	N1093	Nw-0	Neuweilnau, Germany	N1409
Col-4	Landsberg, Poland	N933	Ob-0	Oberursel, Germany	N1419
Ct-1	Catania, Italy	N1095	Per-1	Perm, Russia	N1445
Cvi-0	Cape Verdian Islands	N902	Pi-0	Pitztal/Tirol, Austria	N1455
Db-0	Dombachtal, Germany	N1101	Pla-0	Playa de Aro, Spain	N1459
Dijon	Dijon, France	N919	Pog-0	Point Grey, Canada	N1477
Dra-0	Drahonin, Czech Republic	N1117	Rak-2	Raksice, Czech Republic	N1485
Edi-0	Edinburgh, England	N1123	Rld-1	Netherlands	N913
Ei-2	Eifel, Germany	N1125	Rsch-0	Rschew, Russia	N1491
El-0	Ellershausen, Germany	N1135	S-96	Netherlands	N914
En-1	Enkheim, Germany	N1137	Sap-0	Slapy, Czech Republic	N1507
Er-0	Erlangen, Germany	N1143	Sav-0	Slavice, Czech Republic	N1515
Est	Estonia	N911	Stw-0	Stobowa, Russia	N1539
Fi-0	Frickhofen, Germany	N1157	Tsu-1	Tsu, Japan	N1640
Fr-2	Frankfurt, Germany	N1169	Ty-0	Taynult, England	N1573
Ga-0	Gabelstein, Germany	N1181	Wc-1	Westercelle, Germany	N1589
Gd-1	Gudow, Germany	N1185	Wei-0	Weiningen, Switzerland	N3110
Gö-0	Göttingen, Germany	N1195	Wil-1	Wilna, Russia	N1595
Gr-1	Graz, Austria	N1199	Ws-0	Wassilewskija, Russia	N1603
Gü-0	Gückingen, Germany	N1213	Ws-1	Wassilewskija, Russia	N2223
Gy-0	La Minière, France	N1217	Ws-4	Wassilewskija, Russia	N5390
Ha-0	Hannover, Germany	N1219	Wt-4	Wietze, Germany	N1610
Hl-0	Holtensen, Germany	N1229			

0.5 mM $MgCl_2$, 50 μM NaFe-EDTA, 2.5 mM MES, 50 μM H_3BO_3 , 12 μM $MnCl_2$, 1 μM $CuCl_2$, 1 μM $ZnCl_2$, 30 mM NH_4Mo , Bactoagar 8 g L^{-1} , adjusted to pH 5.7 with 1 M KOH and autoclaved at 120 °C for 20 min. After sowing, the plates were cold-treated at 4 °C for 24 h and subsequently placed in a near vertical position in a culture room: 20 °C, 70% relative humidity, 16 h light (250 $\mu mol m^{-2} s^{-1}$) using high pressure sodium lamps (Vialox[®] Nav-T 400 Super; Osram Ltd, Langley, Berkshire, UK) and metallic halogens lamps (Powerstar[®] HQI-BT 400 D; Osram Ltd). At day 6, the plantlets were either maintained under normal culture conditions, by transfer into Petri dishes containing CM and 1 mM KH_2PO_4 , or phosphate-deprived, by transfer into Petri dishes containing CM supplemented with 1 mM KCl. For phosphate-deprived plants, it should be pointed out that, due to the slight phosphate content of Bactoagar, the CM contained approximately 1 μM phosphate. Accordingly, this medium will hereafter be termed 'low-phosphate medium'. The Petri dishes were scanned daily using a flat-bed scanner (300 dpi).

Measurement of growth parameters

Images corresponding to different growth times (T) were analysed using Optimas[®] software version 6.1 (Media Cybernetics, Silver Spring, MD, USA). The projected leaf area (A_L) and the total root length (L_{TR}) were automatically detected on the corresponding region of the picture. The primary root length (L_{PR}), the branched zone length (L_{BZ}) and the lateral root number (N_{LR}) were manually determined.

Data were then exported to a spreadsheet to calculate additional parameters: the total lateral root length (L_{LR}): $L_{LR} = L_{TR} - L_{PR}$; the lateral root density (D_{LR}): $D_{LR} = N_{LR}/L_{PR}$; the mean distance between lateral roots (DI_{LR}): $DI_{LR} = L_{BZ}/N_{LR}$; the primary root growth rate (GR_{PR}): $GR_{PR} = \Delta L_{PR}/\Delta T$; and the average lateral root growth rate

(GR_{LR}): $GR_{LR} = \Delta L_{LR}/\Delta T$. All parameters corresponded to the mean value from seven plants.

Statistical analysis

All results were statistically analysed using the Statistica[®] software (Statsoft, Tulsa, OK, USA). For the analysis of variance, two and three factor analysis of variance (ANOVA) with a LSD *post-hoc* test were used for assessing differences ($P = 0.05$), respectively, at day 14 (A_L , L_{TR} , L_{PR} , L_{LR} , L_{BZ} , N_{LR} , D_{LR} and DI_{LR}) and in temporal analysis (GR_{PR} , GR_{LR} and N_{LR}).

RESULTS

Current accessions display different architectures and variations in their response to phosphate availability

Previous work about the effects of phosphate availability on *Arabidopsis* growth parameters concerned mainly the architecture of the root system (Williamson *et al.* 2001; Linkohr *et al.* 2002; Lopez-Bucio *et al.* 2002; Al-Ghazi *et al.* 2003) and was nearly exclusively performed using the *Columbia* accession. In order to delineate those effects of general importance in *Arabidopsis*, we first selected a small subset of accessions (Col, Cvi, Ler and Ws) that are widely used to generate mutant collections or RILs. Figure 1 compares 2-week-old-plantlets from the four accessions Cvi-0, Col-4, Ler-1 and Ws-1, when cultured under normal medium (1 mM phosphate) or low-phosphate medium (1 μM). Quite large differences in the architecture of the root system can be seen (Fig. 1). In addition, a low phosphate availability seemed to induce contrasting responses between accessions (Fig. 1).

The distribution of growth between roots and leaves (Fig. 2, left), and the effects of phosphate availability on this distribution (Fig. 2, right), were first investigated by

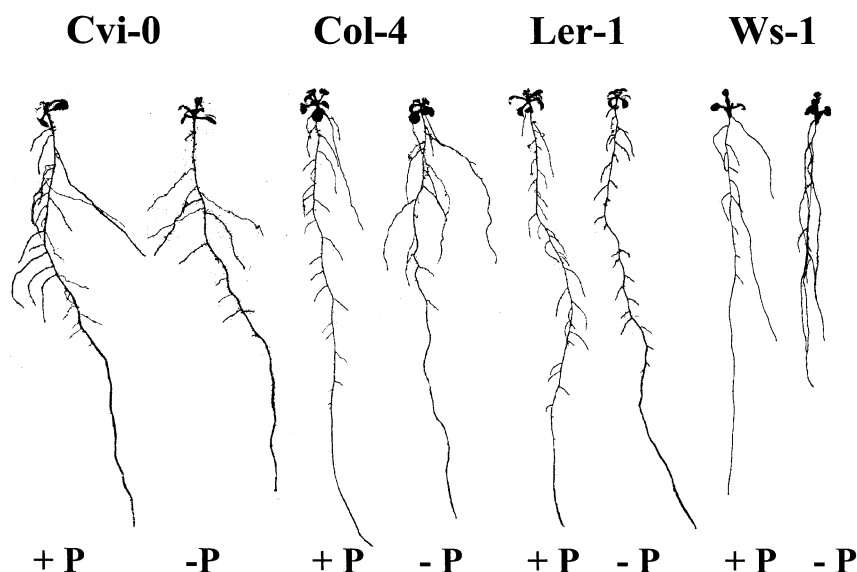


Figure 1. Root system architecture of current *Arabidopsis* accessions. Plants were grown for 2 weeks either on normal culture medium (+ P, 1 mM phosphate) or on low-phosphate medium (- P, 1 μM phosphate).

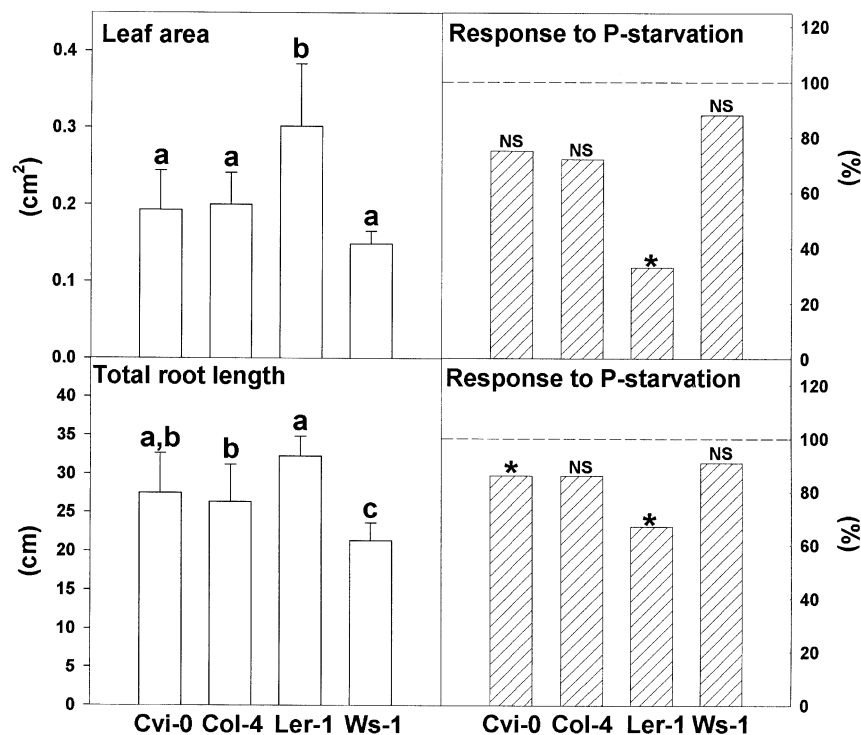


Figure 2. Effects of phosphate availability on plant growth. The relative leaf area and total root length were measured at day 14. Left: parameters values for control plants cultured on normal culture medium; letters and errors bars represent groups that differed significantly and confidence intervals ($P = 0.05$), respectively. Right: changes induced by P-starvation, normalized to the corresponding control; non-significant (NS) and significant (*) responses at $P = 0.05$. All results were the average value from seven seedlings.

global image analysis of the plants. In terms of leaf area (Fig. 2, top), the four accessions were found to fall into two groups: accessions Cvi-0, Col-4 and Ws-1 showed similar leaf area ($A_L \sim 0.18 \text{ cm}^2$ at day 14), which was significantly smaller than that of Ler-1 ($A_L \sim 0.3 \text{ cm}^2$) when grown in normal medium. At the same time, only this latter was significantly affected by P availability, and the projected leaf area decreased by 60% in phosphate-starved Ler-1. A slightly more complex pattern was observed under normal conditions concerning the total length of the root system (Fig. 2, bottom). In this case, differences were observed between accessions Col-4, Ler-1 and Ws-1, but Cvi-0 differed significantly only from Ws-1. However, when the plants were grown in low-phosphate medium, a significant decrease in the total root length was only observed with accessions Cvi-0 and Ler-1.

Detailed characterization of growth in 2-week-old current accessions

Taken together, the observations above suggested that more natural variation was to be expected in root development than in leaf growth. In order to get more details concerning variations in root growth, the total root length was decomposed into three parameters: the primary root length, the total lateral root length and the length of the branched zone (Fig. 3). Primary root length was a highly consistent parameter, showing only little variability (Fig. 3, top panel). Accordingly, although extreme values differed by less than 20% ($L_{PR} \sim 9.6 \text{ cm}$ for Cvi-0 and Ws-1 and $\sim 11.2 \text{ cm}$ for Ler-1), significant differences were detected between accessions Cvi-0 and Ws-1 and the two others, as

well as between Col-4 and Ler-1. For all accessions, phosphate starvation significantly reduced the length of the primary root; the highest effect (44%) concerning the Ws-1 accession. Very similar features (Fig. 3, middle panel) were observed for the length of the branched zone, whatever the culture conditions used (the length of the branched zone accounted to about 50–60% of the primary root length for plants cultured under normal conditions, and was reduced by 20–35% in phosphate-starved plants). By contrast, the total length of lateral roots showed both large differences between accessions (ranging between $\sim 12 \text{ cm}$ for Ws-1 and $\sim 21 \text{ cm}$ for Ler-1, at day 14) and a relatively complex distribution (Fig. 3, bottom panel). Accessions Cvi-0 and Col-4 displayed similar behaviour, Cvi-0 being in addition not discernible from Ler-1. On the other hand, Ws-1 had shorter lateral roots. However, for most accessions, P-starvation had no significant effect on the lateral root length. The only exception concerned Ler-1 in which L_{LR} for P-starved plantlets was nearly half that of control.

In addition to length parameters, another crucial feature of the root system architecture concerns the distribution of lateral roots along the primary root. This was investigated by measuring the number of lateral roots and further calculating their density and the mean distance between lateral roots. It was found that the number of lateral roots at day 14 displayed up to a two-fold variation between accessions (Fig. 4, top panel). Moreover, both the number of lateral roots and the lateral root density distinguished two accessions from the two others (Fig. 4, top and middle panels), and phosphate starvation promoted a significant decrease in both the number and density of laterals for most accessions. By contrast, the

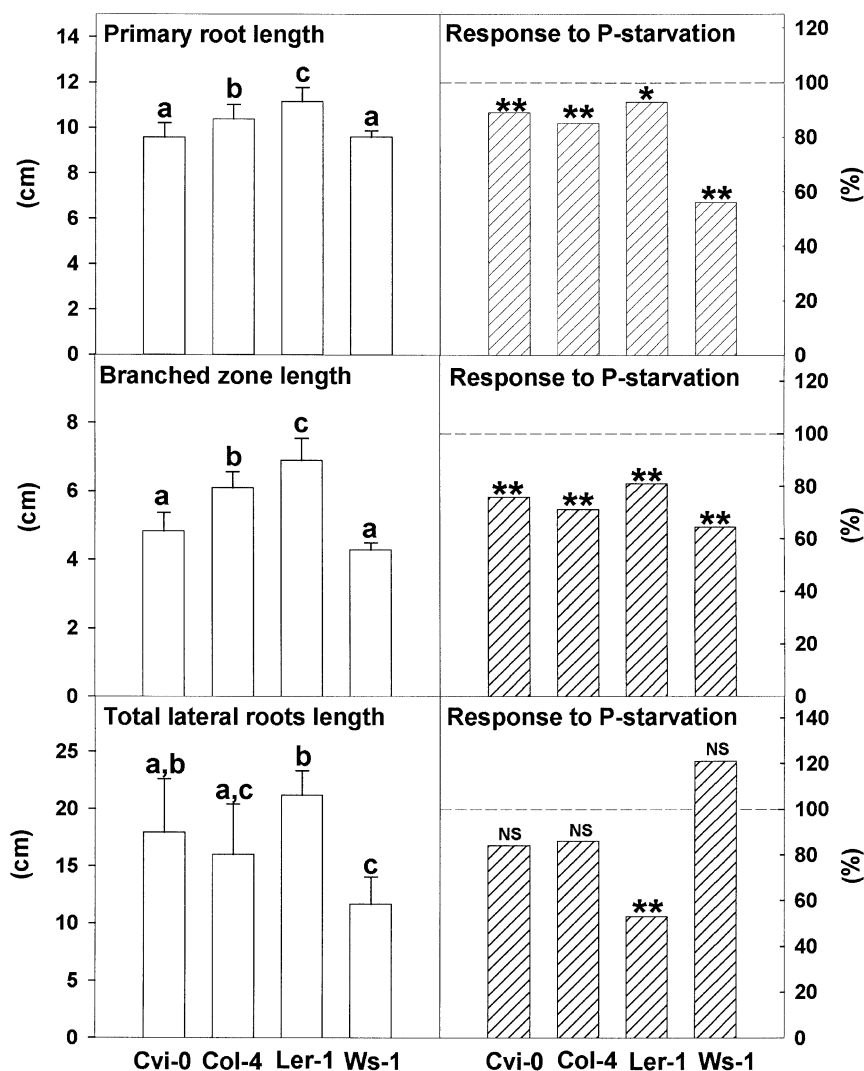


Figure 3. Effects of phosphate availability on root length parameters. The primary roots length, total lateral root length and branched zone length were measured at day 14. Left: parameters values for control plants cultured on normal culture medium; letters and errors bars represent groups that differed significantly and confidence intervals ($P = 0.05$), respectively. Right: changes induced by P-starvation, normalized to the corresponding control; non-significant (NS) and significant (*) responses at $P = 0.05$; highly significant (**) responses ($P = 0.01$). All results were the average value from seven seedlings.

mean distance between lateral roots was very accession-dependent and remained unaffected by phosphate starvation (Fig. 4, bottom panel).

Effect of phosphate availability on time-related growth parameters

The results above suggested that, for 2-week-old plantlets, length parameters for the primary root and the number of lateral roots strongly discriminated the four accessions, especially with respect to the effects of phosphate availability. However, it can not be excluded that variations in the overall plant development rate could occur between accessions and this would affect the simple comparison of plantlets at only one age. Therefore, a complementary analysis was performed on a temporal basis. In a first step, markers for development were researched on plants grown under normal condition by calculating the time required for arbitrarily selected parameters, such as a 4-cm length for the primary root or the emission of five lateral roots (Fig. 5). In both cases, significant differences

were observed, Ler-1 showing the fastest development and Ws-1 the slowest one. Therefore, a more detailed analysis was performed from day 7 on, using plants grown up to that time on normal medium. When maintained under normal conditions, all the accessions displayed changes with time in the growth rate of the primary root (Fig. 6, full symbols). With the exception of Col-4, they elongated first with a continuously increased growth rate. In addition, at the end of the period, all accessions slowed their elongation, this event occurring earlier for Ler-1. This biphasic behaviour disappeared in the low-phosphate medium, with the exception of Ler-1 (Fig. 6, open symbols), whereas the three other accessions showed highly significant differences in their time-course of elongation. Completely different features were observed for the elongation of lateral roots (Fig. 7). In this case, elongation rates displayed no decrease with time (Fig. 7, full symbols). In addition, they were less sensitive to phosphate availability and a significant effect of phosphate availability on the time-course of elongation was observed only for Ler-1 (Fig. 7, open symbols).

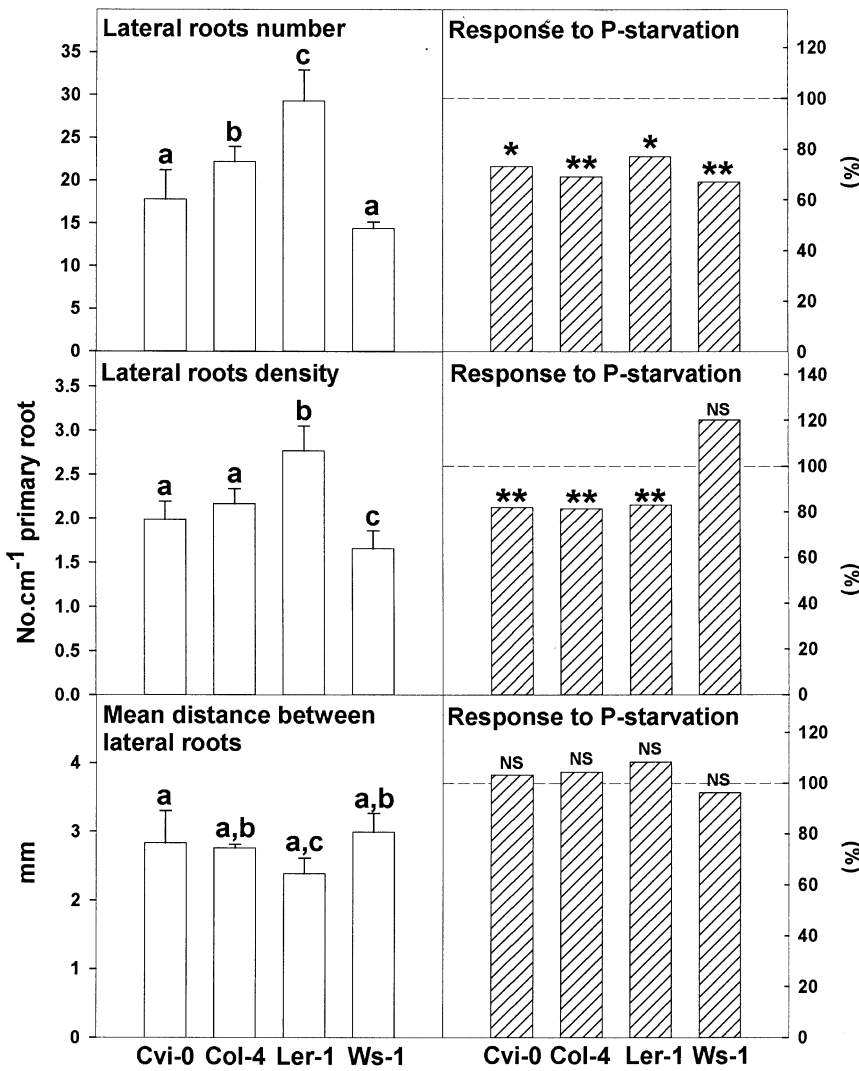


Figure 4. Effects of phosphate availability on lateral root parameters. The lateral root number, lateral roots density and mean distance between lateral roots were measured at day 14. Left: parameters values for control plants cultured on normal culture medium; letters and errors bars represent groups that differed significantly and confidence intervals ($P = 0.05$), respectively. Right: changes induced by P-starvation, normalized to the corresponding control; non-significant (NS) and significant (*) responses at $P = 0.05$; highly significant (**) responses ($P = 0.01$). All results were the average value from seven seedlings.

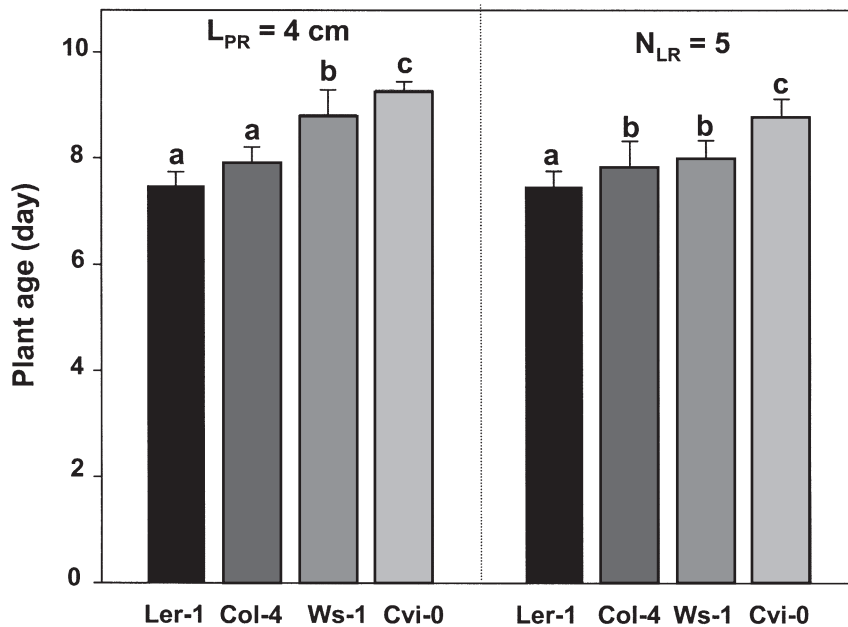


Figure 5. Effects of natural variation between accessions on markers of the rate of development. Developmental markers were estimated by calculating the time required to observe a 4-cm primary root (left) and five lateral roots (right). Letters and errors bars represent groups that differed significantly and confidence intervals ($P = 0.05$), respectively. All results were the average value from seven seedlings.

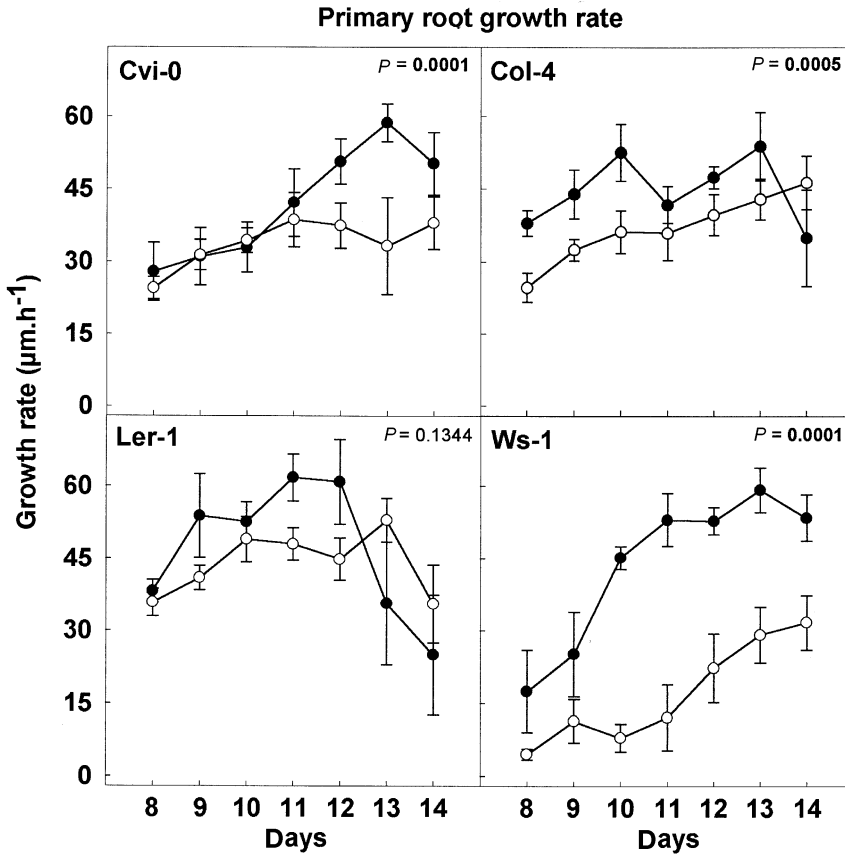


Figure 6. Effects of phosphate availability on the primary root growth rate. Plants were grown either on normal culture medium (full symbols) or on low-phosphate medium (open symbols). All results were the average value (\pm SD) from seven seedlings ($P = 0.05$). The probability for a difference between the entire time-courses are at the right top of each panel, and shown in bold for significant differences.

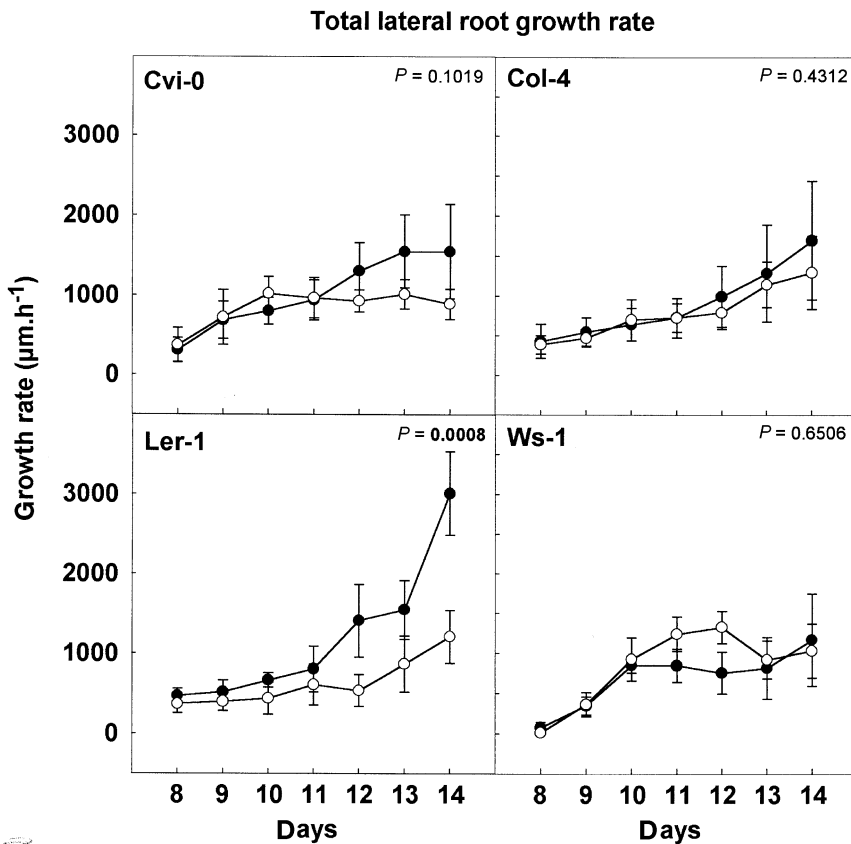


Figure 7. Effects of phosphate availability on the average lateral root growth rate. Plants were grown either on normal culture medium (full symbols) or on low-phosphate medium (open symbols). All results were the average value (\pm SD) from seven seedlings ($P = 0.05$). The probability for a difference between the entire time-courses are at the right top of each panel, and shown in bold for significant differences.

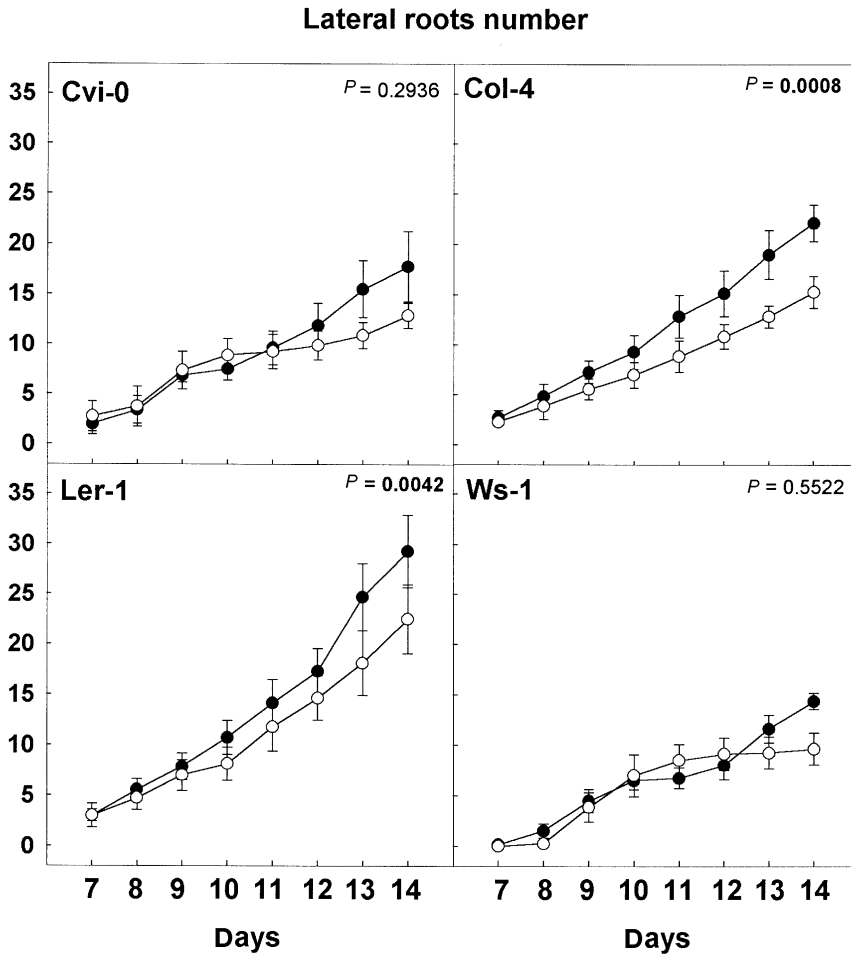


Figure 8. Effects of phosphate availability on lateral root production. Plants were grown either on normal culture medium (full symbols) or on low-phosphate medium (open symbols). All results were the average value (\pm SD) from seven seedlings ($P = 0.05$). The probability for a difference between the entire time-courses are at the right top of each panel, and shown in bold for significant differences.

A similar characterization was made concerning the evolution of the number of lateral roots with time (Fig. 8). For all four accessions, this number was found to increase nearly linearly with time, suggesting that the production rate of lateral roots was almost constant over the analysed period (Fig. 8, full symbols). In addition, the corresponding time-courses were affected by phosphate availability only for Ler-1 and Col-4 (Fig. 8, open symbols), suggesting a lowering of the production rate of lateral roots in the low-phosphate medium.

Large-scale comparison between accessions

Taken together, the time-based results above confirmed that, generally speaking, more pronounced responses to phosphate availability were observed at the level of primary roots in comparison with the lateral roots. Simultaneously, both the opposite behaviour of Ler-1 for the elongation rates and the contrasted responses in terms of production rates of lateral roots suggested that different adaptative strategies could be found among accessions. In order to obtain further insights concerning this hypothesis, a large-scale comparison was undertaken between avail-

able accessions, on the basis of the two parameters showing the most robust response to phosphate availability, the primary root length and the lateral root number. For this purpose, a set of 73 accessions originating from a wide range of habitats was selected (Table 1).

After 2-week culture, 50% of the accessions were found to show both a decrease in the length of primary roots and a reduced number of lateral roots in response to phosphate starvation, similarly to the four previous accessions (Fig. 9, full circles). Within this group, the greatest effect was observed for the accession Er-0 in which P starvation promoted a more than two-fold reduction of each parameter. This group contained in addition the commonly used Col-0 accession. However, one-quarter of the accessions did not appear to be sensitive to phosphate availability (Fig. 9, open circles). On the other hand, the low-phosphate medium only affected some accessions for one of the two parameters (Fig. 9, grey symbols), either the length of primary roots (16% of accessions) or the number of lateral roots (9% of accessions). Extreme behaviours within this group were displayed by accessions Rld-1 and Be-0 which, in response to low phosphate availability, only decreased either the length of the primary root or the number of lateral roots. Moreover, among this latter group, two acces-

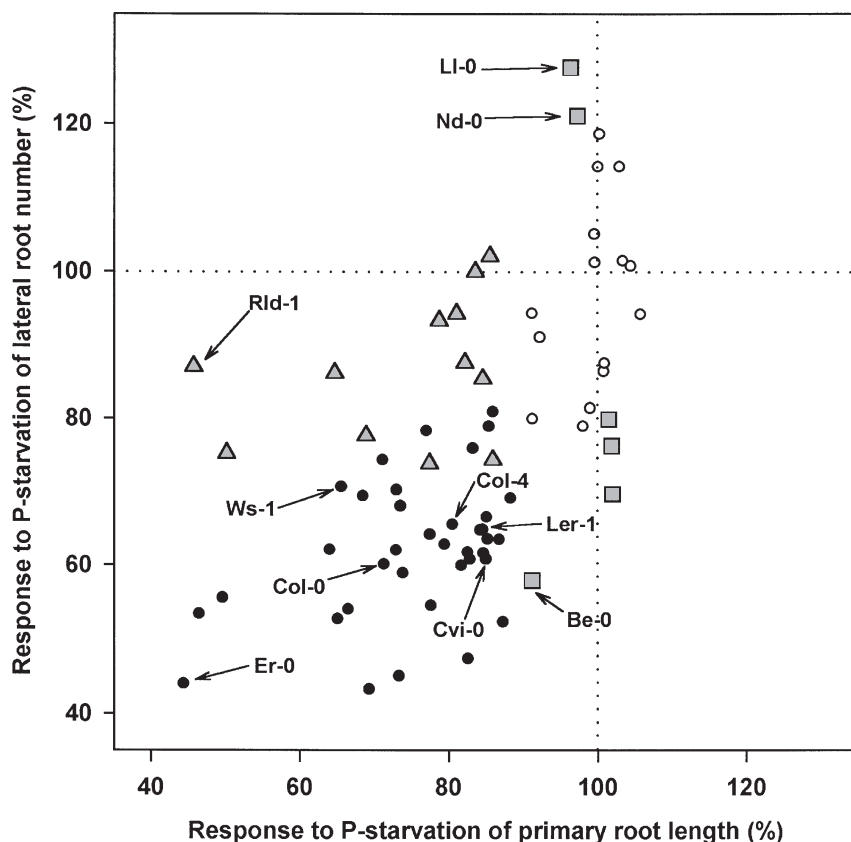


Figure 9. Variations in the phosphate effects between accessions. The effects of phosphate starvation on the length of the primary root and the number of lateral roots were measured for 73 *Arabidopsis* accessions at day 14, and normalized to the corresponding values under control conditions. Full and open circle symbols represent accessions showing significant and non-significant responses, respectively, for both parameters ($P = 0.05$). Grey symbols represent accessions showing a significant response for only one of the two parameters (triangles, primary root length; squares, lateral root number). Accession numbers refer to Table 1.

sions (LI-0 and Nd-0) displayed statistically significant but opposite responses in terms of lateral root number, namely an over-production of lateral roots in response to P-limitation. The root system architecture of these latter accessions is illustrated in Fig. 10.

DISCUSSION

In recent years, a renewed interest in understanding of the growth responses to phosphate availability has emerged, using the model plant *Arabidopsis*, particularly at the level

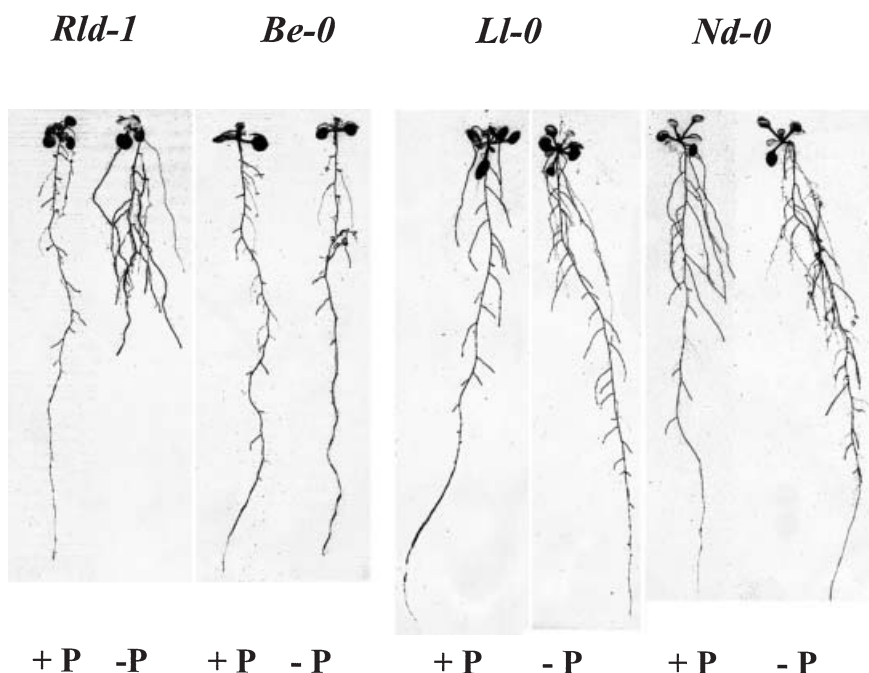


Figure 10. Contrasted strategies for the adaptation of the root system architecture to phosphate availability. Plants were grown as in Fig. 1, either on normal culture medium (+ P, 1 mM phosphate) or on low-phosphate medium (- P, 1 μ M phosphate). Accession numbers refer to Table 1 and Fig. 8.

of hormone signalling (Schmidt & Schikora 2001; Williamson *et al.* 2001; Linkohr *et al.* 2002; Lopez-Bucio *et al.* 2002; Al-Ghazi *et al.* 2003). These studies have taken advantage of both the availability of various mutants and of improvements in non-invasive image acquisition and analysis devices, allowing refined quantitative characterization of the effects of phosphate on plant growth, and particularly on the root system architecture. Nevertheless, in some cases, contradictory results were reported. For instance, phosphate starvation was claimed either to reduce or to increase the number of lateral roots. The origin of such discrepancies is not clear. One tentative explanation is that different accessions could display different adaptive strategies in terms of growth responses to phosphate availability. In the present study, in order to assess this working hypothesis, we decided to compare the effects of phosphate starvation on various *Arabidopsis* accessions. As no growth parameter could be chosen *a priori* to screen the accessions, due to the discrepancies between available results, a three-step strategy was selected. First, the growth of a small number of accessions was characterized in detail. These data were then analysed in order to identify those growth parameters displaying the more discriminant responses to phosphate starvation. Finally a large set of accessions was screened using this limited number of robust parameters. For the first step, we selected the four accessions Cvi-0, Col-4, Ler-1 and Ws-1 because they corresponded to accessions previously selected to generate both recombinant inbred lines (Lister & Dean 1993; Alonso-Blanco *et al.* 1998; Alonso-Blanco & Koornneef 2000) and mutant collections (Bechtold, Ellis & Pelletier 1993; Martienssen 1998; Wisman *et al.* 1998; Tissier *et al.* 1999).

Currently used accessions display large and robust variations in root growth

Quite large variations among accessions concerning the plant size and the leaf number of the rosette have previously been reported in *Arabidopsis* (Karlsson, Sills & Nienhuis 1993; Li, Suzuki & Hara 1998), suggesting that variations could also be expected concerning the leaf surface. Here, by monitoring the growth of aerial parts from the projected leaf area, Ler-1 was indeed found to display a superior growth, but the three other accessions were indistinguishable. As previous parameters describing the growth of aerial parts have been shown to be very sensitive to environmental factors (Karlsson *et al.* 1993), this suggests that our *in vitro* culture conditions would trigger weak variations concerning leaves. Therefore, more attention was given to characterize the root growth in greater detail.

By contrast, much more variation was observed concerning the root system. For the primary root, the two accessions Ws-1 and Cvi-0 showed similar length but differed from both Col-4 and Ler-1. More generally, each of the four length parameters (L_{TR} , L_{PR} , L_{LR} and L_{BZ}) classified the accessions into three significantly different groups, the grouping of accessions depending in addition on the parameters. Similarly, all three parameters used to describe the

contribution of lateral roots to the root system architecture (N_{LR} , D_{LR} and DI_{LR}) also classified the accessions into three other significantly different groups. Furthermore, when looking at growth rate parameters, three kinds of time-course were observed, both for the primary root and total lateral roots. As the parameters included both raw data and derived traits, it is important to note that these variations emerged from all the independently measured parameters (L_{TR} , L_{PR} , L_{BZ} and N_{LR}), indicating an intrinsic diversity. Previous work has already pointed to such variations when considering different accessions. For instance, Krannitz, Aarssen & Lefebvre (1991) compared 25 accessions and showed that the total root length differed by up to 70%, whereas Lopez-Bucio *et al.* (2002) reported smaller differences among four accessions. Therefore, the 50% variation quoted here among four accessions, is in quite good agreement with published data, allowing for the differences in culture conditions. Similar conclusions can be derived for other parameters (such as the primary root, lateral root lengths or lateral root density) from comparisons with the data of Lopez-Bucio *et al.* (2002). Finally, in time-course studies, Beemster *et al.* (2002) demonstrated the occurrence of very large variations for the elongation rate of the primary root (up to 370% at day 9, among a set of 18 accessions grown under continuous light and in the presence of sucrose). In the present work, in which the plants experienced a photoperiod and the medium lacked sucrose, variations below 120% were observed at day 9, but this ratio increased to 380% later on. Therefore, the fact that, although performed under different environmental conditions, all of these studies still led to similar conclusions suggests that the occurrence of very large variations in both the growth and architecture of the root system constitutes a robust response among *Arabidopsis* accessions, in contrast to that observed for leaf growth. In addition, the accessions selected here to illustrate currently used accessions appeared to be also easily distinguishable with respect to root-related features.

Phosphate availability consistently affects the length of the primary root and the number of lateral roots in all the currently used accessions

The effects of phosphate availability were investigated by comparing plants cultured on a sufficient medium (1 mM phosphate) with plants grown on a low-phosphate medium (approximately 1 μ M phosphate). According to phosphate availability, very little variation between accessions was observed for global parameters such as total leaf area or total root length, although, for the four accessions studied, the root length seemed to be more subject to variation than the leaf area. Much more variation emerged when looking at more specific root parameters. For the four accessions, both the length of the primary root, the length of the branched zone and the number of lateral roots were found to be significantly reduced in low-phosphate medium. On the other hand, the time-course of elongation

was affected by phosphate for three of the four accessions in the case of the primary root, but only Ler-1 was sensitive to phosphate level in terms of both total lateral root length and their time-course of elongation. As the total length of lateral roots constitutes an average parameter, it can not be ruled out that significant responses might be observed when measuring the length of individual roots, thus taking into account a possible role of root age. In addition, as the accessions were shown to differ in their overall rate of development, the response to phosphate availability might also simply reflect such differences. However, although both Ler-1 and Col-4 displayed similar and fast growth for the primary root, only Col-4 showed a time-course response that was sensitive to phosphate. A similar disconnection between intrinsic development features and the effect of phosphate availability on time-course responses was observed for the lateral root production rate. Therefore, taken together, all the observations above suggest that the parameters related to the length of the primary root and to the number of lateral roots are among the most discriminant parameters concerning the response to phosphate availability. These conclusions are in full agreement with those from all the previously published studies demonstrating that phosphate starvation reduces the length of the primary root (Williamson *et al.* 2001; Linkohr *et al.* 2002; Lopez-Bucio *et al.* 2002; Al-Ghazi *et al.* 2003). A reduction in the number of lateral roots in low-phosphate medium was also shown in most previous studies (Williamson *et al.* 2001; Linkohr *et al.* 2002; Al-Ghazi *et al.* 2003) with the exception of that by Lopez-Bucio *et al.* (2002).

As, in addition, the measurement of the two parameters L_{PR} and N_{LR} is straightforward and easier to achieve than that of other parameters that also appeared to be sensitive to phosphate availability in all or most accessions, such as the length of the branched zone or the density of lateral roots, L_{PR} and N_{LR} were further selected to screen the behaviour of a large collection of accessions with respect to phosphate availability. In terms of screening strategy, it could be pointed out that the choice to select very discriminant parameters might limit the observation of variations between accessions, and that less stringent parameters could lead to evidence of other variations. In this view, it can not be excluded that other accessions than those identified here could constitute privileged material with which to further investigate the molecular basis of adaptive responses to phosphate starvation. Finally, it should be also emphasized that, for the four accessions cultured under normal medium, a very strong correlation (with coefficients ranging between 0.96 and 0.99) could be calculated between the primary root length and the number of lateral roots. Therefore, the use of two possibly linked parameters could be hypothesized *a priori* to introduce a bias. The subsequent observation that, for one-quarter of the analysed accessions, only one of the two parameters was affected by phosphate starvation suggests that the number of lateral roots is probably not directly linked to the length of the primary root.

Not all accessions display the same adaptive strategy according to phosphate availability

In order to assess the variation between accessions on a larger scale, we selected a set of 73 accessions originating from 18 countries, corresponding mostly to European and American continental stations, in addition to four insular stations. Large amplitudes of variations, by factors of 2.4 and 2.9 for the effects on the primary root length and lateral root number, respectively, were observed for the two measured parameters in response to phosphate availability. In addition, half the accessions displayed the same behaviour as the currently used accessions above, showing that the simultaneous reduction of the growth of the primary root and of the number of lateral roots constitutes a main adaptive response to phosphate starvation in *Arabidopsis*. Interestingly, other accessions showed only one of the two responses. Furthermore, some accessions showed an inverse response. In this way, phosphate starvation appears capable of having either null or negative effects on the primary root, whereas it can have either null, negative or positive effects on the lateral root number. In terms of regulation, these results together suggest that the control of root growth by phosphate availability is not linked to that of branching, although both pathways are likely to share some initial signalling steps. Finally, no clear correlation was found between the geographical origin of accessions and their behaviour.

Up to now, current approaches to elucidate the molecular basis for phenotypic plasticity relied almost exclusively on the search for mutants not displaying the wild-type adaptive response. It can be emphasized that the characterization of clear-cut differential responses between accessions has the capacity to open new routes for the identification of both large-effect genes and favourable allelic variations (Alonso-Blanco & Koornneef 2000). In this respect, several of the accessions studied here could constitute convenient parents to generate populations that would be suitable for the analysis of the developmental plasticity in response to phosphate availability.

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